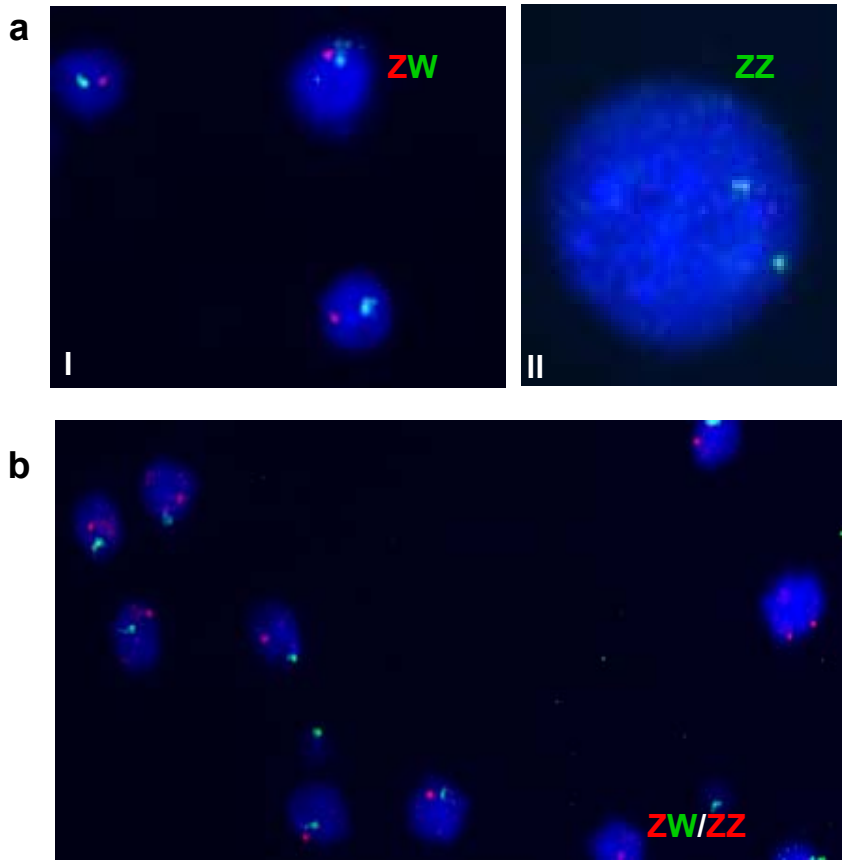
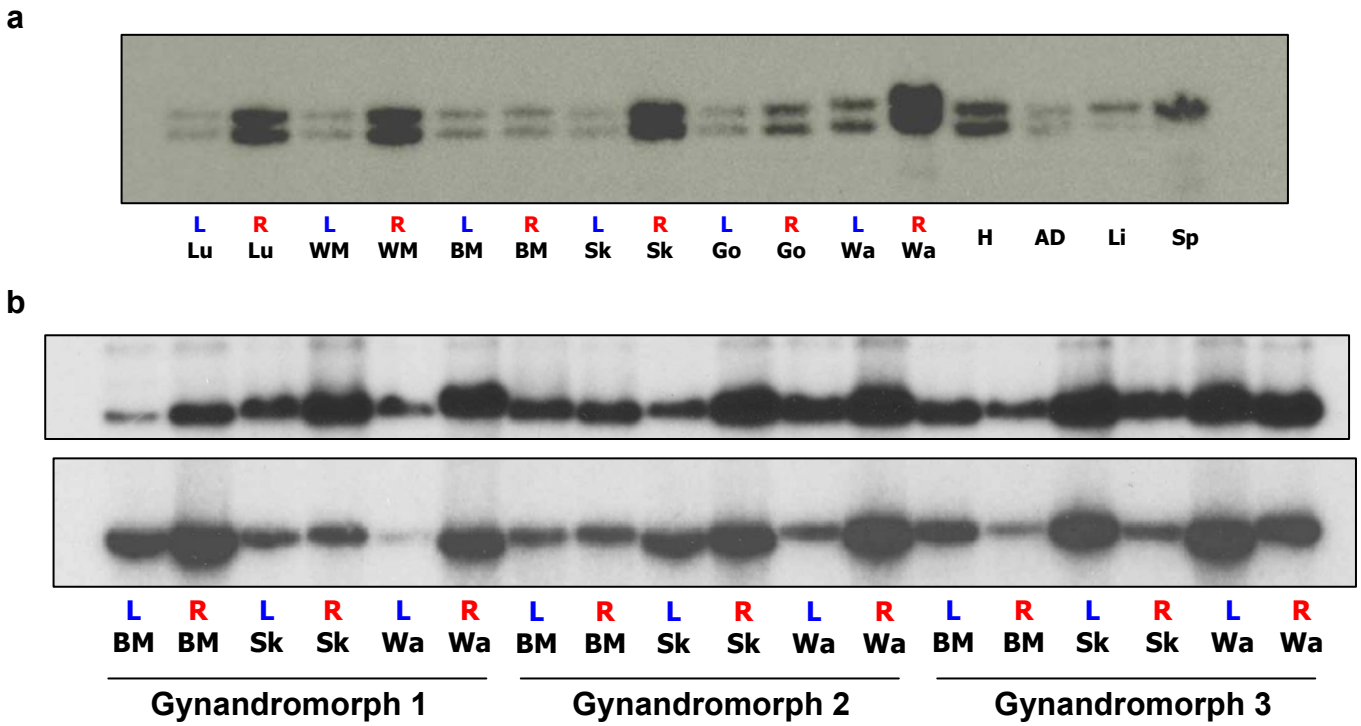
SUPPLEMENTARY INFORMATION

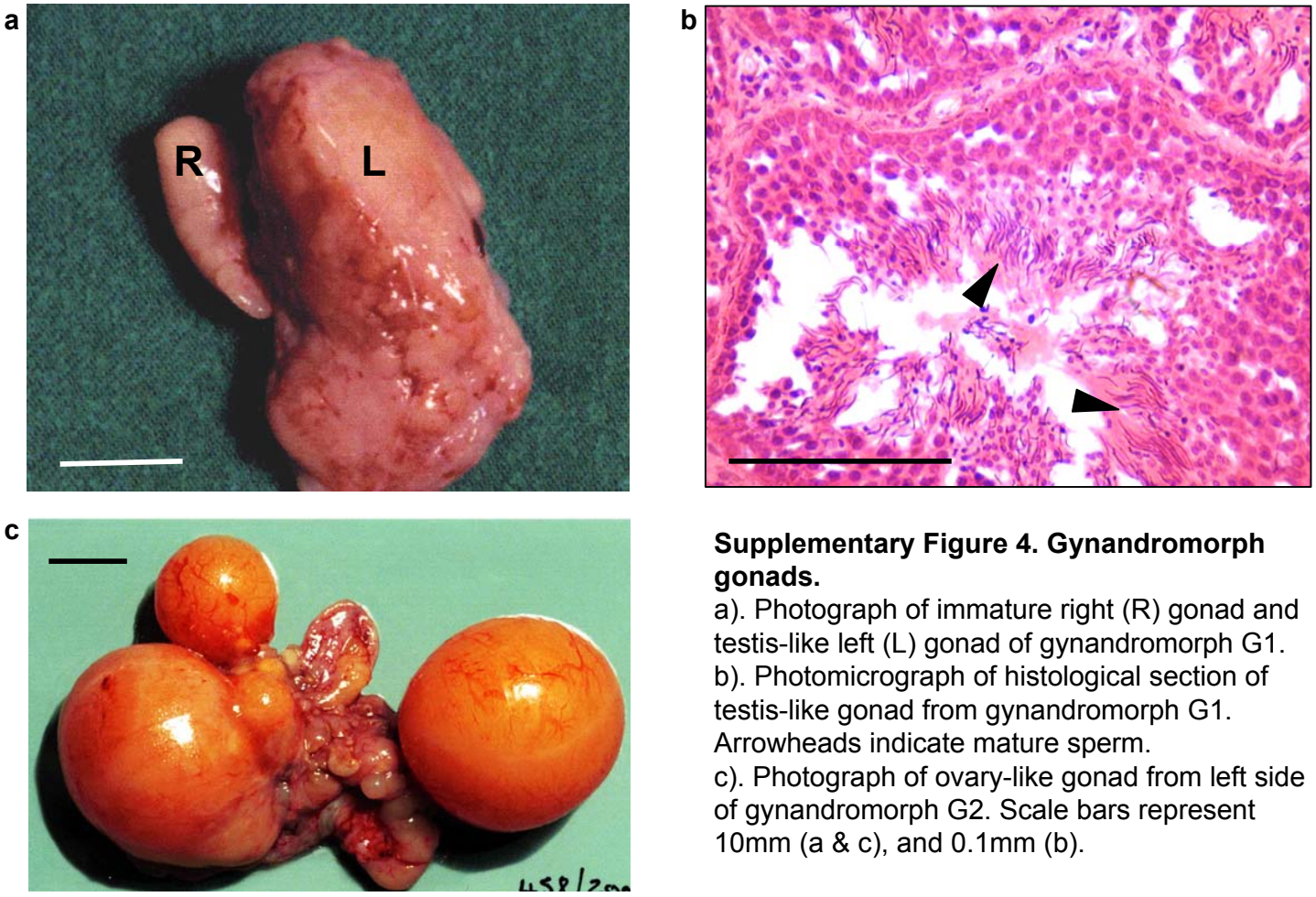
Supplementary Figure 1.
Photographs showing a) right and left sides of gynandromorph birds G1, G2 and G3, and b) typical female and male ISA Brown birds. Although the males occasionally show brown patches on the breast the females are usually uniformly brown. The white patches seen on the brown 'female' side (and *vice versa*) most likely represent 'clones' of male cells. Individual gynandromorphs were housed with two egg laying females and eggs were collected and incubated. None of these eggs proved to be fertile.



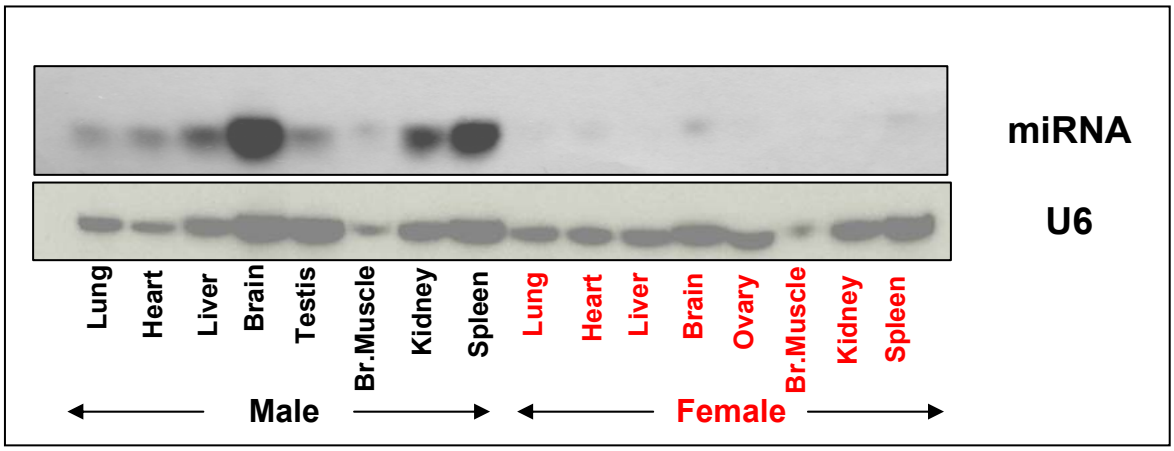
Supplementary Figure 2. FISH analysis of sex chromosomes in blood cells from gynandromorph birds
Interphase nuclei prepared from cultured blood cells from gynandromorph birds G2 (a) and G3 (b) hybridised according to standard FISH procedures with probes specific to both the W- and Z- chromosome (Xho repeat on W- chromosome, and Z-chromosome BAC containing the VLDL receptor gene identified by screening the HGMP chicken BAC library). a) G2 erythrocytes hybridised with probes for Z-chromosome (RED) and W-chromosome (GREEN)(I) and low abundance ZZ G2 leukocyte hybridised with probes for Z-chromosome (GREEN) and W-chromosome (RED) (II). Images from (I) and (II) are presented at similar magnifications.b) G3 blood cells hybridised with probes for W-chromosome (GREEN) and Z-chromosome (RED).



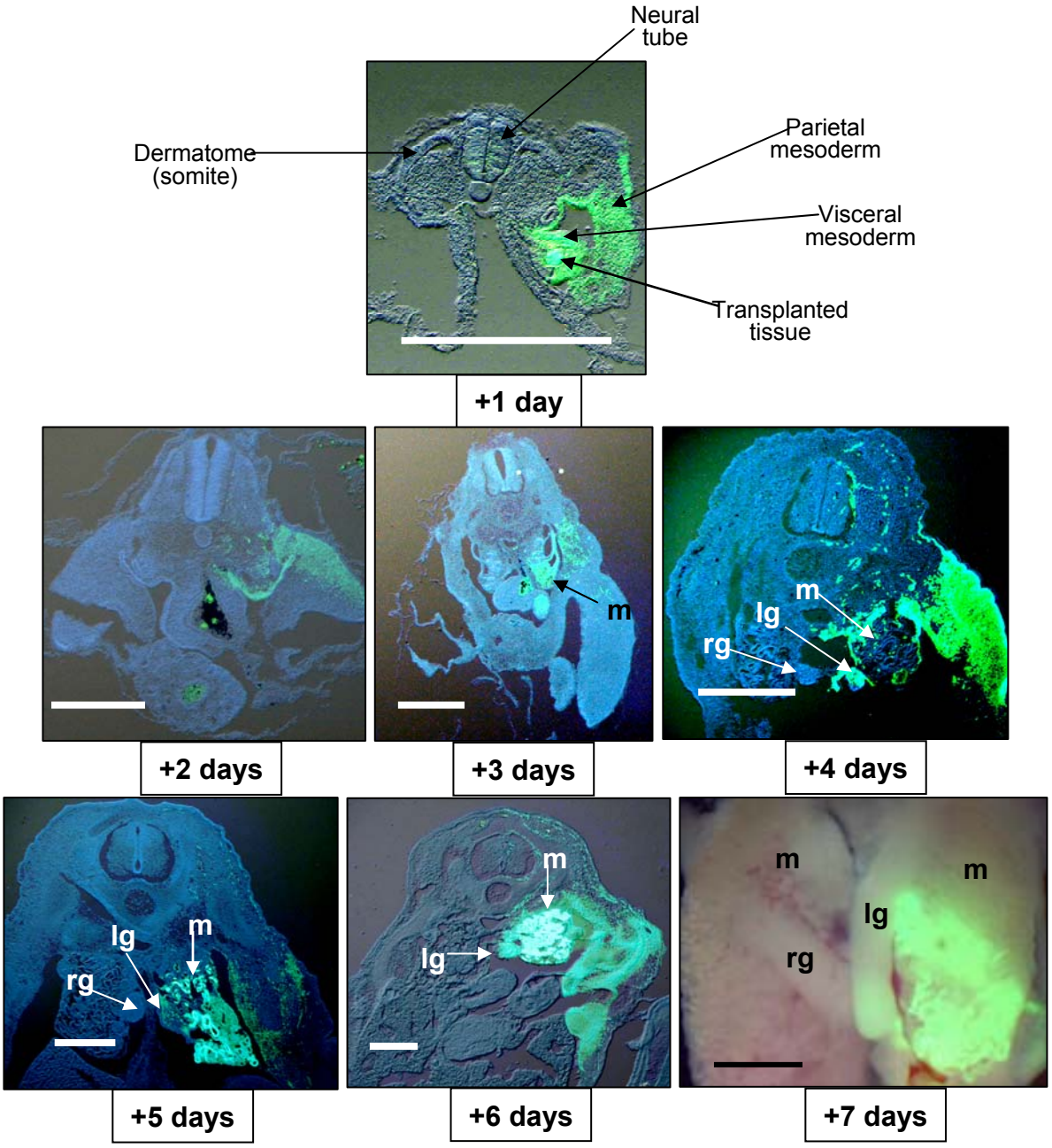
Supplementary Figure 3. Examples of Southern analyses of DNA from tissues from right and left sides of gynandromorph birds.
Equal quantities (2μg) of DNA from individual tissues was hybridised with probe for female specific sequence (W-chromosome gene Faf). Signal intensity reflects relative proportion of W-chromosome containing cells (female) on right and left sides of gynandromorph birds. a) Southern analysis of DNAs from different tissues from gynandromorph bird G1. b) Separate analyses of DNAs from three tissues from right and left sides of gynandromorph birds G1, G2 and G3. L=left, R=right, Lu-lung, WM-wing muscle, BM-breast muscle, Sk-skin, Go-gonad, Wa-wattle, H-heart, AD-accessory duct, Li-liver, Sp-spleen.



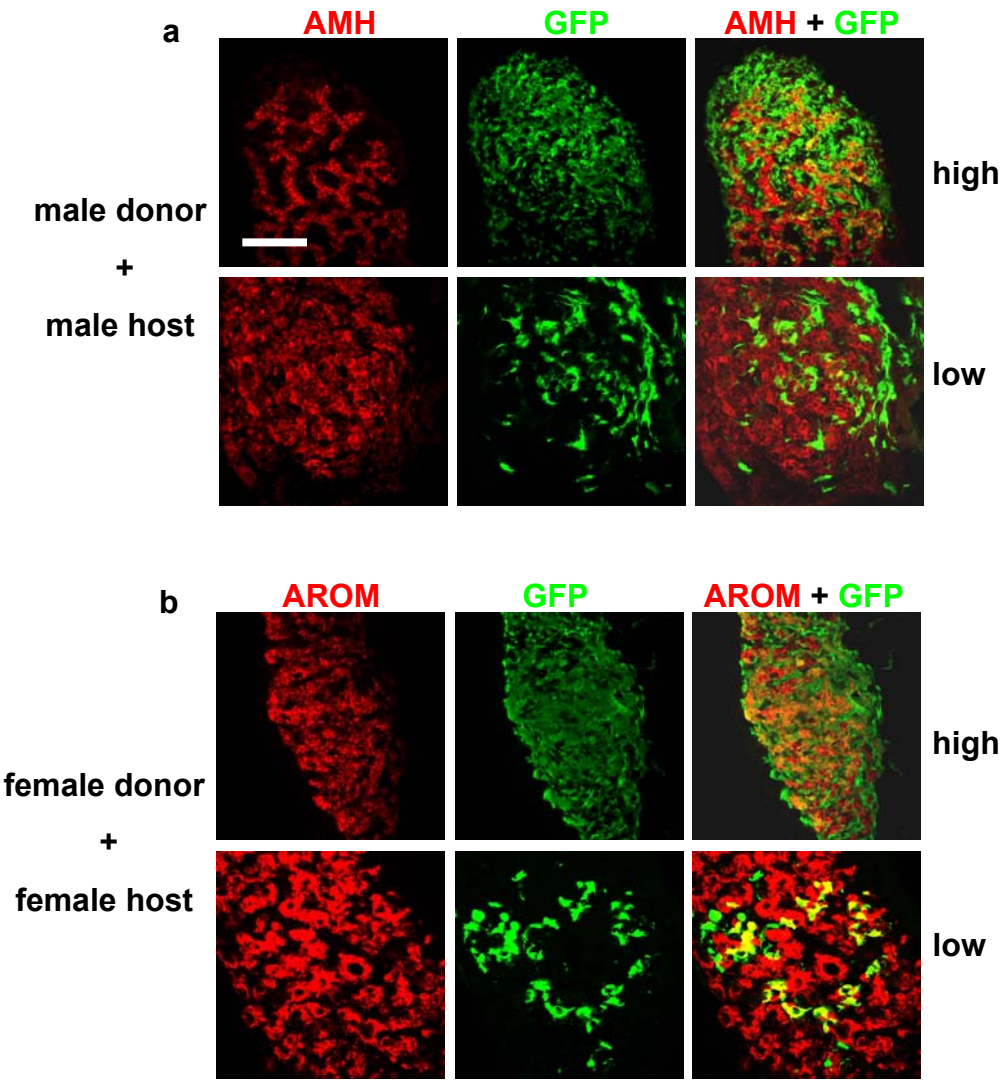
Supplementary Figure 4. Gynandromorph gonads.
a). Photograph of immature right (R) gonad and testis-like left (L) gonad of gynandromorph G1. b). Photomicrograph of histological section of testis-like gonad from gynandromorph G1. Arrowheads indicate mature sperm.
c). Photograph of ovary-like gonad from left side of gynandromorph G2. Scale bars represent 10mm (a & c), and 0.1mm (b).

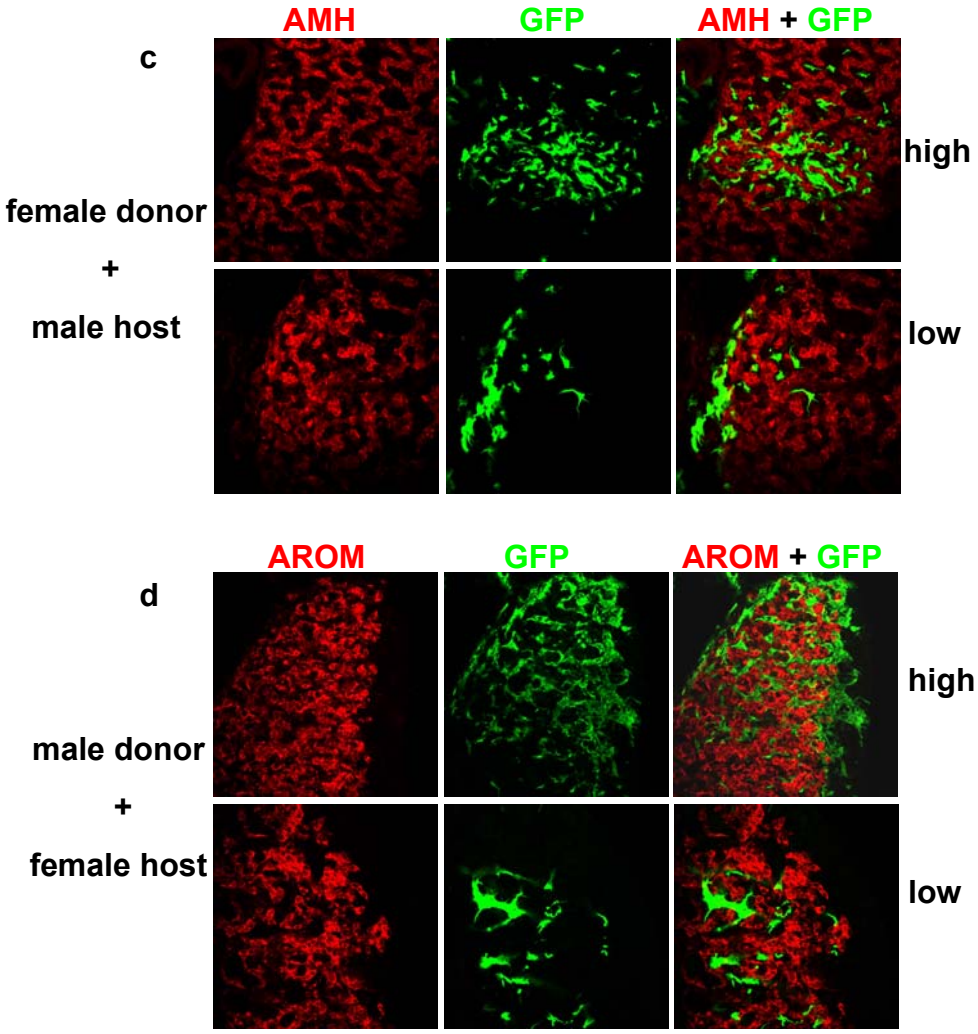


Supplementary Figure 5. **Northern analysis showing expression of novel chicken miRNA in different tissues from male and female adult birds**.
Membrane was hybridised with a LNA probe complementary in sequence to the novel chicken miRNA (gga-mir-2954) and then stripped and re-probed with a sequence complementary to chicken U6-RNA.
Upper panel shows expression of miRNA in male and female tissues and demonstrates that miRNA is expressed at levels 5-10 fold higher in males than in females. Lower panel shows expression of U6 RNA demonstrating similar loading of RNA quantities from equivalent male and female tissues. Quantity (µg) of RNA loaded for each tissue : lung-2, heart-4, liver-7, brain-10, gonads -10, breast muscle-5, kidney-5 and spleen-8



Supplementary Figure 6: Contribution of transplanted donor cells to developing embryo.
Visualisation of GFP-expressing cells between 1 and 6 days following transplantation of donor presumptive mesoderm. GFP-expressing cells are integrated into various tissues including the gonads. Images labelled '+1 to +6 days' show transverse sections through the abdominal region of the embryo. Image labelled '+7 days' shows dissected whole mesonephroi and gonads. m=mesonephros, lg=left gonad, rg=right gonad.


a
AMH
GFP
AMH + GFP
male donor
+
male host
high
low
b
AROM
GFP
AROM + GFP
female donor
+
female host
high
low



Supplementary Figure 7: Contribution of donor cells in same-sex and mixed sex chimeras.
Panels show IHC analysis of sections through chimeric gonads. Donor cells are marked by GFP (green) while AMH and aromatase-expressing cells are shown in red. For each donor:host combination, examples containing a high contribution of donor cells (high) and a low contribution of donor cells (low), are shown. In same sex chimeras (a, b), even examples with a low contribution of donor cells show co-localisation (yellow/orange) of donor cells with functional compartments (AMH/ AROM) of the host gonad, i.e. GFP-expressing cells co-localise with AMH-expressing and aromatase-expressing cells in host testis and ovary respectively. In contrast, in mixed-sex chimeras (c, d), donor cells are not integrated into the functional compartments of the host gonad i.e. no co-localisation of GFP and AMH or aromatase even in examples showing a high contribution of donor cells. Scale bar represents 100 μm.

		G1 (left ♂/right ♀)		G2 (left ♂/right ♀)		G3 (left ♀/right ♂)	
	Side	Value	Ratio*	Value	Ratio	Value	Ratio
Feather colour	right	dark**	-	dark	-	light	-
	left	light**		light		dark	
Wattle (g)	right	1.2	2.4	0.13	4.1	3.3	0.4
	left	2.9		0.54		1.4	
Breast muscle (g)	right	180	1.1	138	1.1	172	0.8
	left	199		150		135	
Leg muscle (g)	right	400	1.2	203	1.2	319	0.8
	left	480		247		259	
Gonad	right	immature testis	-	none	-	immature testis	-
	left	testis-like		ovary-like		ovo-testis	
Femur length (mm)	right	91	1.1	97	1.1	105	0.9
	left	99		107		95	
Femur circumference (mm)	right	73	1.1	73	1.1	78	0.9
	left	81		80		70	
Femur density (mmAl)	right	3.26	1.1	3.09	1.4	7.26	0.5
	left	3.47		4.28		3.50	

Supplementary Table 1. Physical properties of gynandromorph birds

*: Ratio of left side measurements to right side measurements.
**: feathers on the “dark” side were predominantly brown while feathers on the “light” side were predominantly pale.
mmAl = mm of Aluminium.

Sample (days in culture)	Probes	Female (%)	Male (%)
G2-R1 (17)	Z-bio: W-dig	76	24
	Z-dig: W-bio	79	21
G2-R2 (17)	Z-bio: W-dig	48	52
	Z-dig: W-bio	47	53
G2-L (7)	Z-dig: W-bio	75	25
	Z-bio: W-dig	60	40
G3- R (13)	Z-dig: W-bio	48	52
(44)	Z-dig: W-bio	46	54
G3- L (17)	Z-bio: W-dig	94	6
	Z-dig: W-bio	89	11

Table 2a

Bird	ZZ (%)	ZW (%)
G1	53	47
G2	<1*	>99
G3	8	92

Table 2b.

Supplementary Table 2. Proportion of ZW (female) and ZZ (male) cells in cultures derived from skin and blood samples from gynandromorph birds.

Cultured skin cells or circulating blood cells were fixed and prepared for FISH analysis by standard procedures. >100 informative interphase nuclei were hybridised with probes detecting the W and Z- chromosomes (XhoI repeat on W and Z chromosome BACs containing either aldolase B, CHRN, VLDL receptor or SCII genes identified by screening the HGMP chicken BAC library) and were scored in each case.

a. Proportion of ZW (female) and ZZ (male) cells in cultures derived from gynandromorph skin samples.

Skin samples were collected from different sites from the right (R) and left (L) sides of gynandromorph birds (G2 & G3) and were cultured for 7-44 days (as indicated in parenthesis) before fixing and preparing for FISH by standard procedures. Many hybridisations were performed in duplicate with Z and W probes reciprocally labelled with either Biotin-16-dUTP (bio) or digoxigenin 11-dUTP (dig). G2 R1 was prepared from a patch of skin located approximately 2 cm from G2R2.

b. Proportion of ZW (female) and ZZ (male) cells in cultures derived from blood samples of gynandromorph birds (G1, G2 & G3).

The majority of nuclei scored were small erythrocytes but a minority of larger leukocytes were also scored and showed the sex chromosome identity associated with the phenotypic sex identity of the left side of the bird. *Only one such leukocyte was seen from many hundreds of cells analysed for G2.

	Southern analysis	Contribution of female cells (%)					
		Breast		**Skin**		**Wattle**	
		left	right	left	right	left	right
G1 (left♂/right ♀)	I	22	46	18	60	18	57
	II	34	59	18	41	13	32
	III	30	69	15	66	10	68
	IV	20	60	20	76		
	V	27	71	14	42	23	38
	VI	27	60	16	51	27	57
	Mean percentage	**27**	**61**	**17**	**56**	**18**	**50**
G2 (left♂/right ♀)	I	24	42	15	72	37	52
	II	17	28	11	54	22	52
	III			21	66	33	54
	IV	21	53	17	56	25	51
	V	28	50	16	57	23	52
	Mean percentage	**22**	**43**	**16**	**61**	**28**	**52**
G3 (left♀/right ♂)	I	45	26	85	28	74	51
	II	43	13	60	26	80	60
	III	53	15	58	24	69	45
	IV	37	16	67	25	74	52
	V	49	15	64	20	63	23
	VI	41	23	73	26	80	63
	Mean percentage	**45**	**18**	**68**	**25**	**73**	**49**

Supplementary Table 3: Contribution of female cells to individual tissues from right and left sides from three gynandromorph birds.

Tables represent a minimum of four separate Southern analyses on DNA from each gynandromorph bird, e.g. see Figure S3. Membranes were hybridised with probe for W-chromosome sequence.

Phosphorimager values obtained from known quantities of DNA from individual tissues from right and left sides were compared to signals obtained from equivalent quantities of purified female genomic DNA. Results are presented as proportion of individual tissues composed of female cells. Tissues on right side of G1 and G2 contain a higher proportion of female cells than tissues on the left side, whereas in G3, the left side contains the higher proportion of female cells.